IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Sanders et al. Application No.: 10/537,280 Filed: 5/27/2005 Title: Binding Partners for the Thyrotropin Receptor and Uses Thereof Attorney Docket No.: AATH.P-001	Group Art Unit: 1647 Examiner: C.M. Woodward Conf. No.: 1845

SUPPLEMENTAL SUBMISSION

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Supplementing the response to office action filed 10/9/2009 and entered pursuant to the filing of an RCE in this case, Applicants attach a portion of a reply filed in the corresponding European case to the extent that it relates to the Yoshida reference, disclosed in an IDS filed on 10/9/2009. The comments on the Valente paper which also claims to have made certain types of monoclonal antibodies are also included.

As outlined in the attached paper, there are substantial reasons why a person skilled in the art would find the assertions in these papers that monoclonal antibodies with the stated properties had been obtained to be **not credible**. If the Examiner needs this submission in the form of a Declaration under Rule 132, Applicants can of course provide one from the inventors. It is

noted, however, that these conclusions are entirely consistent with the third party praise of the present inventors as the first to actually achieve this result. See Amendment of 6/3/2008, Exs. D and E. Note also that Ex E states, with reference to the antibodies of Yoshida and Valente that "these mAbs did not show any TSH competing activities themselves." (Page 139, left column).

Respectfully submitted,

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Enclosure

5.1 Yoshida *et al* (D5)

In D5, four monoclonal antibodies to the TSH receptor are reported. However, the methods used to isolate these antibodies are such that monoclonal antibodies would not reasonably be expected to result. Therefore, it cannot be concluded that the molecules reported in D5 to be monoclonal antibodies were actually monoclonal antibodies.

For example, it is reported in D5 that samples were obtained from two patients with untreated hyperthyroid Graves' disease. However, the levels of thyroid stimulating activity found in the serum of these patients were low: in the case of the patient used to prepare $T^{R}Mo\text{-}^{2}$, 20mg/ml of serum IgG was found to give 135% stimulation of the TSH receptor (see Table 1) whereas only 1mu/ml of TSH gave 600% stimulation of the TSH receptor (Table 2). It is clear, therefore, that the IgG of this patient was capable of only very low levels of TSH receptor stimulation, even at relatively high concentrations. It is also reported

in D5 that inhibition of labelled TSH binding to the TSH receptor by 20mg/ml of this patient's serum IgG was only 68%. Therefore, it is clear that both the TSH receptor stimulatory and inhibitory activity of this patient's IgG was low. This is significant because when peripheral blood lymphocytes are cultured in the form of isolated cells or after initial fusion with a myeloma cell line (as in D5), the levels of IgG in general and of specific antibodies produced in the cultures are much lower than the levels in the lymphocyte donor patient serum. Consequently, unless the level of specific antibody – in the case of D5, TSH receptor autoantibody – is very high, it is not usually possible to detect the specific antibody in the cultures. Therefore, in view of the low levels of TSH receptor autoantibody in the donor serum in D5 (low levels of the antibody are apparent from the low activity levels reported), the skilled person would not expect monoclonal antibodies to be obtainable from the patients used in D5 and therefore he would view the results reported in D5 with caution.

Lymphocytes were isolated from the peripheral blood of the two patients discussed above. These lymphocytes were fused with human myeloma cells, and then "screened for the presence of anti-TSH receptor antibodies". However, the method actually used to screen the cultures is not described in D5 (any one of the three different procedures mentioned in D5 could have been used). In the absence of these details, it is not possible to tell whether or not monoclonal antibodies have been obtained, nor what their properties are.

It is then reported in D5 that positive clones were grown up on plates and cloned twice by limiting dilution. The cloned cells were grown up in flasks and the supernatants from these cells were tested for the presence of monoclonal antibodies to the TSH receptor. However, again no indication is given of the actual method used to detect the presence of these monoclonal antibodies.

In addition to the above-described problems with D5, there is no mention of expression levels or clone stability in D5. There is also no clear evidence that (a) a stable single clone of hybrid cells has been produced or (b) that the cultures produce actual human monoclonal antibodies. In the absence of this important information, the skilled person on reading D5 would not conclude that human monoclonal antibodies have been produced.

It is reported in D5 that the antibodies obtained were assayed as follows:

(i) inhibition of ^{125}I labelled TSH binding to human thyroid membranes;
(ii) stimulation of cAMP production in human thyroid membranes;
(iii) binding to microwells coated with Triton X-100 solubilised human thyroid membranes detected by ^{125}I-labelled protein A; and
(iv) Western blotting of Triton X-100 extracts of human thyroid membranes, with initial SDS-PAGE run under denaturing conditions (2% SDS and 5% mercantoethanol).